

## CLAIMS

1. A variant of a parent Termamyl-like alpha-amylase, comprising an alteration at one or more of the following positions:

R28, R118, N174; R181, G182, D183, G184, G186, W189, N195, M202, Y298, N299, K302, S303, N306, R310, N314; R320, H324, E345, Y396, R400, W439, R444, N445, K446, Q449, R458, N471, and N484,

wherein

(a) the alteration(s) are independently

(i) an insertion of an amino acid downstream of the amino acid which occupies the position,

(ii) a deletion of the amino acid which occupies the position, or

(iii) a substitution of the amino acid which occupies the position with a different amino acid,

(b) the variant has alpha-amylase activity and

(c) each position is numbered according to the amino acid sequence of SEQ ID NO: 12.

2. The variant of claim 1, which further comprises one or more of the following mutations:

Delta G184; Delta (R181-G182); Delta (D183-G184); R28N,K; S94K; R118K; N125A,R,K; N174D; R181Q,E,K; G186R; W189R,K; N195F; M202L; Y298H,F; N299A; K302R, S303Q, N306G,D,R,K; R310A,K,Q,E,H,D,N; N314D; R320K; H324K; E345R,D,K,N; Y396F; R400T,K; W439R; R444K; N445K,Q; K446N; Q449E; R458K; N471E; N484Q.

3. The variant of claim 1 or 2, which further comprises a mutation in M202.

4. The variant of claim 3, wherein the mutation is M202L,T.

5. The variant of any of claims 1-4, which further comprises a mutation in N195.

6. The variant of claim 5, wherein the mutation is N195F.

7. The variant of any of claims 1-6, which further comprises a mutation in G186.

8. The variant of claim 7, wherein the mutation is G186R.

9. The variant of any of claims 1-8, which further comprises a mutation in R181.

5 10. The variant of claim 9, wherein the mutation is R181Q.

11. The variant of any of claims 1-10, wherein the parent Termamyl-like alpha-amylase is derived from a strain of *B. licheniformis*, *B. amyloliquefaciens*, *B. stearothermophilus*, *Bacillus* sp. NCIB 12289, NCIB 12512, NCIB 12513 or DSM 9375, or DSMZ no. 12649, KSM AP1378, or  
10 KSM K36 or KSM K38.

12. The variant of any of claims 1-11, wherein the parent Termamyl-like alpha-amylase has an amino acid sequence of SEQ ID NOS: 2, 4, 6, 8, 10, 12, or 13.

13. The variant of any of claims 1-11, wherein the parent Termamyl-like alpha-amylase has an amino acid sequence which has a degree of identity to SEQ ID NO: 4 of at least 60%, preferably 70%, more preferably at least 80%, even more preferably at least about 90%, even more preferably at least 95%, even more preferably at least 97%, and even more preferably at least 99%.  
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14. The variant of any of claims 1-11, wherein the parent Termamyl-like alpha-amylase is encoded by a nucleic acid sequence, which hybridizes under low, preferably medium, preferred high stringency conditions, with the nucleic acid sequence of SEQ ID NO: 11.

25 15. The variant of any of claims 1-14, which variant has altered solubility, preferably increased solubility, in particular under washing, dish washing or hard surface cleaning conditions.

16. A DNA construct comprising a DNA sequence encoding a variant of any of claims 1-15.

30 17. A recombinant expression vector which carries a DNA construct of claim 16.

18. A cell which is transformed with a DNA construct of claim 16 or a vector of claim 17.

19. A cell of claim 18, which is a microorganism, preferably a bacterium or a fungus, in particular a gram-positive bacterium, such as *Bacillus alkalophilus*, *Bacillus amyloliquefaciens*, *Bacillus brevis*, *Bacillus circulans*, *Bacillus coagulans*, *Bacillus lautus*, *Bacillus lentus*, *Bacillus licheniformis*, *Bacillus stearothermophilus*, *Bacillus subtilis*, or *Bacillus thuringiensis*.

20. A composition comprising a variant of any of claims 1-15.

21. A detergent additive comprising a variant of any of claims 1-15, optionally in the form of a non-dusting granulate, stabilised liquid or protected enzyme.

22. A detergent additive of claim 21, which contains 0.02-200 mg of enzyme protein/g of the additive.

23. A detergent additive of claim 21 or 22, which additionally comprises another enzyme such as an amylase or another amylolytic enzyme, such as glucoamylase, cellulase, CGTase, lipase, maltogenic amylase, mannanase, peroxidase, or protease.

24. A detergent composition comprising a variant of any of claims 1-15 and a surfactant.

25. A detergent composition of claim 24, which additionally comprises another enzyme such as another amylolytic enzyme, a cellulase, CGTase, lipase, maltogenic amylase, mannanase, peroxidase, or protease.

26. A manual or automatic dishwashing detergent composition comprising a variant of any of claims 1-15.

27. A dishwashing detergent composition of claim 26, which additionally comprises another enzyme such as an amylase or another amylolytic enzyme, such as glucoamylase, cellulase, CGTase, lipase, maltogenic amylase, mannanase, peroxidase, or protease.

28. A manual or automatic laundry washing composition comprising a variant of any of claims 1-15.

29. A laundry washing composition of claim 28, which additionally comprises another enzyme such as an amylase and/or another an amylolytic enzyme, such as glucoamylase, cellulase, CGTase, lipase, maltogenic amylase, mannanase, peroxidase, or protease.

30. Use of a variant of any of claims 1-15 or a composition of any of claims 20-29 for washing and/or dishwashing.

31. Use of a variant of any of claims 1-15 or a composition of any of claims 20-29 for textile desizing.

32. Use of a variant of any of claims 1- 15 or a composition of any of claims 20-29 for starch liquefaction.

33. A method of producing a variant of a parent alpha-amylase having an altered property relative to the parent, wherein the parent alpha-amylase has the sequence of SEQ ID NO: 2, 4, 6, 8, 10, 12 or 13, or has a sequence at least 60 % identity to said sequence, said method comprising:

(a) modelling the parent alpha-amylase on the three-dimensional structure of SEQ ID NO: 4 depicted in the Appendix 1 to produce a three-dimensional structure of the parent alpha-amylase;

(b) identifying in the three-dimensional structure obtained in step (a) at least one structural part of the parent wherein an alteration in said structural part is predicted to result in said altered property;

(c) modifying the sequence of a nucleic acid encoding the parent alpha-amylase to produce a nucleic acid encoding a deletion, insertion, or substitution of one or more amino acids at a position corresponding to said structural part; and

(d) expressing the modified nucleic acid in a host cell to produce the variant alpha-amylase;

wherein the variant has alpha-amylase enzymatic activity and has at least one altered property relative to the parent.

34. The method of claim 33, wherein the altered property is selected from the group consisting of substrate specificity, substrate binding, substrate cleavage pattern, temperature

stability, pH dependence of enzymatic activity, pH dependence of stability, stability towards oxidation,  $\text{Ca}^{2+}$ -dependency, specific activity or altered solubility, preferably increased solubility.

35. A method of constructing a variant of a parent alpha-amylase having an altered property relative to the parent, wherein the parent alpha-amylase has the sequence of SEQ ID NOS: 2, 4, 6, 8, 10, 12 or 13 or has a sequence at least 60 % identity to said sequence, said method comprising:

(a) modelling the parent alpha-amylase on the three-dimensional structure of SEQ ID NO: 4 depicted in the Appendix 1 to produce a three-dimensional structure of the parent alpha-amylase;

(b) comparing the three-dimensional structure obtained in step (a) with a three-dimensional structure of an unrelated alpha-amylase, wherein the unrelated alpha-amylase differs from the parent alpha-amylase in said property;

(c) identifying a structural part of the three-dimensional structure obtained in step (a) which is different from the three-dimensional structure of the unrelated alpha-amylase and which is predicted to be relevant to said property;

(d) modifying the sequence of a nucleic acid encoding the parent alpha-amylase to produce a nucleic acid encoding a deletion, insertion, or substitution of one or more amino acids at a position corresponding to said structural part; and

(e) expressing the modified nucleic acid in a host cell to produce the variant alpha-amylase;

wherein the variant has alpha-amylase activity and has one or more altered properties as compared to the parent alpha-amylase.

36. The method of claim 35, wherein, in step (d), the modification is a deletion, substitution, or insertion of one or more amino acid residues in the parent alpha-amylase so that the structural part in the variant corresponds to the structural part in the unrelated alpha-amylase.

37. A method of increasing the solubility of polypeptide crystals, in particular enzyme crystals, wherein one or more amino acid residue(s)

1) located within a distance of 6.0 Å of a neighbouring enzyme molecule in the tertiary crystal structure, and

2) interacting with said neighbouring enzyme molecule,  
are mutated.

38. The method of claim 37, wherein the amino acid residues to be mutated are located with  
5 3.5 Å of a neighbouring enzyme.

39. The method of claim 37 or 38, wherein the enzyme has amylase, carbohydrase,  
CGTase, glucoamylase, lipase, lyase, mannanase, maltogenic amylase, mannanase,  
oxidoreductase, protease, or transferase, activity.

40. The method of claim 37, wherein the amylase is an alpha-amylase, such as Termamyl-  
like amylase.

41. The method of any of claims 37-40, wherein the polypeptide is a protein having  
5 biological activity.

42. The method of any of claims 37-41, wherein the polypeptide has antimicrobial activity.

43. The method of any of claims 37-42, wherein the polypeptide is selected from the group  
20 comprising: insulin, growth hormone, EPO, TPO, Factor VII, Factor VIII.

44. A method of increasing the solubility of Termamyl-like alpha-amylase crystals, wherein  
one or more amino acid residue(s)

1) located within a distance of 6.0 Å of a neighbouring Termamyl-like alpha-amylase  
25 molecule in the tertiary crystal structure, and

2) interacting with said neighbouring Termamyl-like alpha-amylase molecule, are  
mutated.

45. The method of claim 44, wherein the amino acid residues to be mutated are located with  
30 3.5 Å of a neighbouring Termamyl-like alpha-amylase.

46. The method of claim 44 or 45, wherein the crystal structure of SP722 depicted in APPENDIX 1 is used as a reference for determining the distance between neighbouring alpha-amylases.

47. The method of any of claims 44-46, wherein the crystal structure is a structure modelled in accordance with any of claims 26-29 from of SP722 depicted in APPENDIX 1 is used as a reference for determining the distance between neighbouring alpha-amylases.

48. The method of any of claims 44-47, wherein the Termamyl-like alpha-amylase include amylases selected from the group of alpha-amylase derived from a strain of *B. licheniformis*, *B. amyloliquefaciens*, *B. stearothermophilus*, *Bacillus* sp. NCIB 12289, NCIB 12512, NCIB 12513 or DSM 9375, or DSMZ no. 12649, KSM AP1378, K38, K36.

49. The method of claim 48, wherein the Termamyl-like alpha-amylase is any of the alpha-amylases selected from the group depicted in SEQ ID NOS: 2, 4, 6, 8, 10, 12, and 13.

50. The method of any of claims 44-49, wherein the Termamyl-like alpha-amylase has an amino acid sequence which has a degree of identity to SEQ ID NO: 4 of at least 60%, preferably 70%, more preferably at least 80%, even more preferably at least about 90%, even more preferably at least 95%, even more preferably at least 97%, and even more preferably at least 99%.

51. The method of any of claims 44-50, wherein the Termamyl-like alpha-amylase is encoded by a nucleic acid sequence, which hybridizes under low, preferably medium, preferred high stringency conditions, with the nucleic acid sequence of SEQ ID NO: 11.

52. The method of any of claims 44-51, wherein the Termamyl-like alpha-amylase amino acid residues being less than 6.0 Å from the nearest neighbouring amylase molecule are the following (using SP722 numbering):

19, 20, 21, 22, 25, 28, 29, 53, 76, 84, 87, 90, 93, 94, 124, 125, 126, 128, 142, 144, 156, 157, 158, 159, 160, 161, 170, 171, 172, 173, 174, 175, 183, 184, 185, 186, 187, 188, 189, 190, 193, 195, 196, 197, 209, 212, 226, 229, 256, 257, 258, 259, 280, 281, 298, 299, 300, 302, 303, 304,

305, 306, 310, 311, 314, 319, 320, 321, 322, 341, 345, 405, 406, 408, 444, 447, 448, 449, 463, 464, 465, 466, 467.

53. The method of any of claims 44-51, wherein the Termamyl-like alpha-amylase amino  
5 acid residues being less than 3.5 Å from the nearest neighbouring amylase molecule are the following (using SP722 numbering):

22, 25, 28, 76, 94, 125, 128, 158, 160, 171, 173, 174, 184, 189, 209, 226, 229, 298, 299, 302, 306, 310, 314, 320, 345, 405, 447, 466.